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COMPARISON OF TWO CULTURES FOR USE IN PREACIDIFIED SKIMMILK FOR COTTAGE CHEESE MANUFACTURE

BY

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COMPARISON OF TWO CULTURES FOR USE IN PREACIDIFIED SKIMMILK FOR COTTAGE CHEESE MANUFACTURE

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Thesis Adviser Date

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Head, Dairy Science Department Date

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Curd formation in a cultured dairy product is brought about by the addition of a bacterial culture capable of producing lactic acid from lactose. The primary function of this culture is to produce sufficient lactic acid to cause coagulation of the caseinate system of skimmilk. A secondary function of the culture is the production of flavor compounds considered to be desirable in these products. The flavor compounds produced by the normal growth of citric acid fermenting organisms are water soluble, and are largely removed during washing of the Cottage cheese curd. Therefore, flavor compounds are normally added to the cheese in the creaming mixture.

Because of the many factors involved in the preparation and use of lactic starter or culture bacteria, it would be advantageous to develop a procedure relying less on the lactic starter for production of lactic acid. The acid producing function of a starter can be replaced by the direct addition of acid to skimmilk. In doing so, the milk must be acidified in such a way that a definite curd structure is developed. The possibility of direct addition of an acidulant to skim milk has been explored by several research workers in the industry. This procedure does not make use of equipment presently available in the industry and as yet the product does not meet the Federal Standards of Identity for Cottage cheese.

The purpose of this research was to modify the present short set Cottage cheese making procedure by the simultaneous use of a

chemical acidulant and lactic starter to shorten the make time of Cottage cheese and increase product uniformity and quality.

Since very little previous work has been done in this area, this investigation was undertaken to determine if preacidification of skimmilk for Cottage cheese production is feasible from economic and quality standpoints. In evaluating the feasibility of preacidification, the acid producing ability of cells, the increase or decrease in coagulation time, and the tension of the curd were the properties of particular interest.

REVIEW OF LITERATURE

Insufficient or slow acid production by lactic starter bacteria has long been a problem in the manufacture of cultured dairy products. Differences in flavor, lack of uniformity and poor keeping quality are some additional problems resulting from the poorly controlled use of cultures. Investigators have long felt a satisfactory product could be developed by using a chemical acidifying process. The possibility that starter bacteria might be eliminated in the manufacture of cultured dairy products became apparent about 1954, following studies conducted by Ernstrom (10) in a cooperative research effort with the University of Wisconsin and the Creamery Package Manufacturing Company. This project developed a commercially practical, continuous process for production of a Cottage cheese like product.

Cottage cheese is defined as a product containing not less than 80% moisture; and, in addition, creamed Cottage cheese must contain not less than 4% milk fat (9). The curd is formed from pasteurized skim milk primarily by acid developed by lactic streptococci. The lactic streptococci currently used in the industry for acid production are Streptococcus lactis and Streptococcus cremoris. These organisms may appear as single strains or in combinations as mixed strain lactic cultures. The quantity of these cultures used is determined by the manufacturing procedure employed. When the acidity has developed to approximately pH 4.70, the coagulum is cut

with wire knives to form cubes ranging from ¼-1 inch in size. Following cutting, the curd is generally allowed to remain undisturbed for 15 minutes or longer. The curd particles are then slowly "cooked" for 1-2 hours to 50-60 C. Slow uniform heating is very important. When the curd is at the desired firmness, heating is stopped and the whey is drained. The curd is washed 2 or 3 times with cool water to chill the curd and reduce the sharp acid flavor. After the wash water is removed, the curd may be salted lightly and refrigerated.

Small amounts of rennet are often used in Cottage cheese production at the option of the manufacturer (12). Lochry (16), in 1949, experimented with the use of rennet for the manufacture of Cottage cheese. One milliliter of rennet was added for each 1,000 pounds of skimmilk in combination with 0.5-1% active starter. This concentration of rennet was equivalent to an increase of 20-30 times the coagulation properties of the coagulator now in general use in the industry.

Consumer preferences for Cottage cheese. Tuckey (27) in 1957 reported that Cottage cheese consumption since 1946 had increased faster than any other dairy product. He attributed the increase to improved product quality and an increased appreciation of Cottage cheese, by the consuming public, as an economical and nutritious food. The Milk Industry Foundation (19) reported an increase of 1.6 pounds of Cottage cheese consumed per capita from 1950 to 1965.

This increase of 1.6 pounds raised the total per capita consumption to 4.7 pounds. Cottage cheese accounted for 1/3 of all the cheeses produced in the United States in 1965.

Angevine (1,2) believed the bland, mild acid and clean flavored type Cottage cheese had increased consumption of Cottage cheese in western and midwestern states. He stated that the great increase in Cottage cheese consumption can only be maintained by a more uniform quality and an extension of the shelf-life of the product.

Consumer preferences for Cottage cheese were conducted by Olson and Strozier (21). The surveys indicated preferences for acidity were over a rather wide range, with cheese high in acid (pH about 5.0) preferred over low acid cheese (pH above 5.2). The consumer opinions of Cottage cheese indicated a rather high regard for the product. The principal objections to Cottage cheese were that it had poor keeping quality and lacked flavor. In the past, consumers of Cottage cheese and other cultured dairy products have expected a strong flavor. The Cottage cheese was frequently sour, and at times it had developed fermented and/or yeasty flavors. To insure consumer preferences only fresh, good quality skimmilk should be used in the manufacture of Cottage cheese, and all steps in the manufacturing process must be scientifically controlled.

Many advances have been made in improving Cottage cheese quality. Factors such as improved equipment, modern control methods, good refrigeration and better handling have made it possible to produce

fairly uniform and mild-flavored Cottage cheese. Consumers of Cottage cheese quickly notice any flavors, either absorbed or chemically produced, and any off-flavors produced by undesirable bacteria. If such defects occur, consumption of Cottage cheese immediately decreases unless these defects are not corrected.

Tuckey (28) stressed that freshness is still a premium factor in terms of quality in Cottage cheese. One must consider that the time has increased between actual processing and the time the cheese reaches the consumer. The primary factors increasing the time between processing and consumption are the tendency to centralize processing in areas away from urban populations and the tendency to leave the product in the store longer, before removing it as a product loss. Economic factors force processors and store personnel to follow these procedures and there is very little hope that these conditions will be changed in the future.

Direct acidification in products other than Cottage cheese. The use of direct acidification in Cottage cheese manufacture has led research workers to develop the direct acidification process for other dairy products.

Shehata and Olson (22) developed a method of manufacturing Blue cheese by direct acidification. They reported that Blue cheese formed by adjusting the pH of milk to 5.60, by the addition of acids before coagulation with rennet, was similar in body and texture to curd made by traditional procedures. The direct

acidification method offered certain economies over the traditional method. Manufacturing time and the amount of rennet used were reduced by 50%, whereas the amount of starter was reduced by 75%.

Breene et al. (4) developed a procedure for the manufacture of Pizza cheese without starter. The cheese possessed satisfactory flavor and when baked it possessed excellent melting and stringing properties. The cheese was made from pasteurized milk containing 2% fat. The milk was acidified at 5 C to pH 5.60 with either lactic, acetic, or hydrochloric acid and set at 37-38 C with 100 milliliters of rennet per 1,000 pounds of milk. The curd was cut with 1/4-inch knives, held at the setting temperature for 80 minutes, heated to 50 C for 5 minutes, drained, molded, and brine-salted. The pH at which the curd was molded was higher (pH 5.60) than in conventional procedures using lactic starter (pH 5.30-5.40). Since there was no lactic acid produced by fermentation the pH remained the same in the finished cheese. These procedures for the manufacture of rennet type cheese differ from conventional methods in that continued acid production during manufacture and curing does not occur. Some workers think that the rate of acid production is an essential part of manufacturing these cheeses.

Direct acidification has been successfully used in other fermented dairy products besides cheese. Deane and Thomas (7) reported success in the use of chemical acidulants to replace cultures in the manufacture of sour cream. The cream was standardized to 18-19% milk fat, pasteurized at 80 C for 30 minutes, homogenized at 2,000

pounds per square inch and then cooled. Lactide, a high melting isomer of lactone, and glucono-delta-lactone were acidulants used to coagulate the cream. The lactide was difficult to dissolve and produced a product with a lumpy body and astringent flavor. Glucono-delta-lactone, however, dissolved readily and produced a sour cream with a smooth texture and slightly weak coagulum. A slight astringent flavor developed in 7-8 hours when the product was held at 24 C or above. Viscosity was increased by adding 0.20-0.25% stabilizer or 2% nonfat dry milk. A combination of glucono-delta-lactone and 1% of a 10% citric acid solution added in an amount equal to 9% of the weight of the total serum solids present in the cream produced a product without noticeable astringent flavor. Deane and Thomas (7) used starter distillate, added in amounts up to 2 ounces per gallon of cream, to produce a product with a definite culture aroma. The flavor of the product, although less intense, resembled that of cultured cream.

Direct acidification in Cottage cheese manufacture. The only instance of complete curd formulation by a chemical acidulant, other than an acid, was proposed by Deane and Hammond (6) in 1959. These investigators worked with a number of compounds which slowly hydrolyze to produce acids suitable for Cottage cheese making.

The compounds tested included anhydrides, esters, lactones, and lactides. From these compounds D-glucono-delta-lactone and racemic-lactide were the most suitable for the formation of a milk coagulum.

D-glucono-delta-lactone, at a concentration of 12% of the solids-not-fat in the skimmilk, produced a final pH of 4.6-4.7 after cooking. Coagulation time varied from 15-16 hours at 20 C. A temperature of 35 C lowered the coagulation time to 3 hours. Setting temperatures above 40 C caused immediate matting after cutting of the curd and were not considered acceptable for quality cheese production. A racemic-lactide level of 9% of the solids-not-fat level, gave a coagulation time of approximately 2 hours at 25 C and 1 hour at 30 C.

The pH at cutting was dependent upon the rate of hydrolysis of the compound which, in turn, was dependent upon the temperature.

One cannot predict to what extent this process will replace those using a starter culture. It does not appear likely that the savings in time, labor, equipment, and culture would offset the added costs of the acidogen compounds. However the acidogen process would not be affected by the presence of antibiotics or bacteriophage particles. The ease of control and duplication of results should make this process easily adaptable to automated cheese making.

A completely automated Cottage cheese making apparatus was proposed by Lankford (14) in 1960. In this process, milk and starter culture were fed through a vertical, jacketed cylinder in which the pH of milk was decreased to near the isoelectric point (pH 4.70). The mixture was elevated to the top of a vertical coagulation

cylinder where a proteolytic enzyme was metered into the milk. This mixture was allowed to trickle onto a cone-shaped distributor before entering the cylinder in which coagulation took place. The newly formed curd mass was then pushed downwards by gravity through a wire mesh screen; where the curd was cut by a vertical knife and fed into a horizontal jacketed cooking cylinder. The curd particles were then distributed onto a mesh screen conveyor belt where provisions were made for spray washing of the curd. Lankford (14) noted that this apparatus could also be easily adapted to continuous Cheddar cheese manufacture.

Leeder et al. (15) were the first to investigate the combined use of acid and starter culture for the manufacture of Cottage cheese. They proposed that a shortening of the manufacturing time could be accomplished in this way. The most desirable concentration of milk solids, kind and amount of acidulant, and size of inoculum were determined before the investigation began. Hydrochloric acid inhibited the starter bacteria the least and accounted for a greater reduction in setting time than any of the other acids tested. Ten to twelve percent milk solids was the most desirable concentration of solids used in the reconstituted milk. A pH of 5.40 was the limiting range of acidification, and acidification to this pH resulted in a 20-25% reduction in setting time. Acidification of the skimmilk 5-10 minutes before adding starter gave the most desirable results with a 15% inoculum.

Yields of curd and curd losses in whey were found by Morgan et al. (20) to be unaffected by increasing the rate of inoculation of a vat from 5 to 20%. Increased inoculum was suggested as a means of reducing the setting time and giving added protection from bacteriophage attacks. Lord and Olson (17) in 1963 reported that an increase in lactic starter from 5% to 10% adversely affected the body and texture of Cottage cheese. Leeder (15) reported that Cottage cheese made by the acid and lactic culture procedure could be of comparable quality to that made by the conventional method.

Lord and Olson (17) experimented with the acidification of water used to reconstitute nonfat dry milk. The use of reconstituted milk of low pH, together with 10% culture, reduced manufacturing time about 50% and gave a Cottage cheese of satisfactory quality. The body and texture of the Cottage cheese was slightly improved when fresh skimmilk was fortified with nonfat dry milk.

McNurlin (18) in 1962 developed a procedure of direct addition of acid to skimmilk for the formation of curd for Cottage cheese manufacture. The addition of concentrated lactic or hydrochloric acid to skimmilk at 5 C, followed by warming without agitation to 21-27 C, resulted in a firm curd suitable for Cottage cheese. The amount of acid needed was determined by acidifying a small volume of milk at 21 C to the desired pH, and adding a proportional amount to the cheese milk. Babel (3), in his study on Cottage cheese cultures, proposed that coagulation must occur in a quiescent state. He believed the addition of a chemical acidulant could not be a

satisfactory substitute for acid produced by a bacterial culture. McNurlin (18) proposed the use of electrical-resistance heating to warm the milk without agitation, which allowed coagulation to take place in a quiescent state.

Ernstrom (11), in 1954, developed a process for curd formation by replacing the acid-producing function of a culture starter with direct addition of acid to cold milk. At 5 C it was possible to add concentrated acid to milk and adjust the pH to 4.70-4.50 without causing coagulation. The amount of acid to be added was determined by acidifying a small aliquot of milk to the desired pH and adding a proportionally larger amount to the bulk milk. With this procedure, it was possible to establish the acidity at the exact level desired for curd formation. Skimmilk will coagulate readily at pH 4.70 when the temperature is at or about 21 C. Therefore, the cold acidified milk, when warmed without agitation to about 21 C, formed a smooth curd suitable for Cottage cheese. Satisfactory curd was formed with lactic acid, citric acid, phosphoric acid, acetic acid, hydrochloric acid, and sulfuric acid. Hydrochloric acid was most satisfactory from a cost standpoint and no particular problem was observed in connection with body and texture of the Cottage cheese when the solids content of the skimmilk was between 8 and 12%.

On the basis of information provided by Ernstrom (11), the Creamery Package Manufacturing Company built an apparatus they called a curd former. The principle of operation of the curd former is illustrated diagrammatically in Figure 1. A vertical tube having

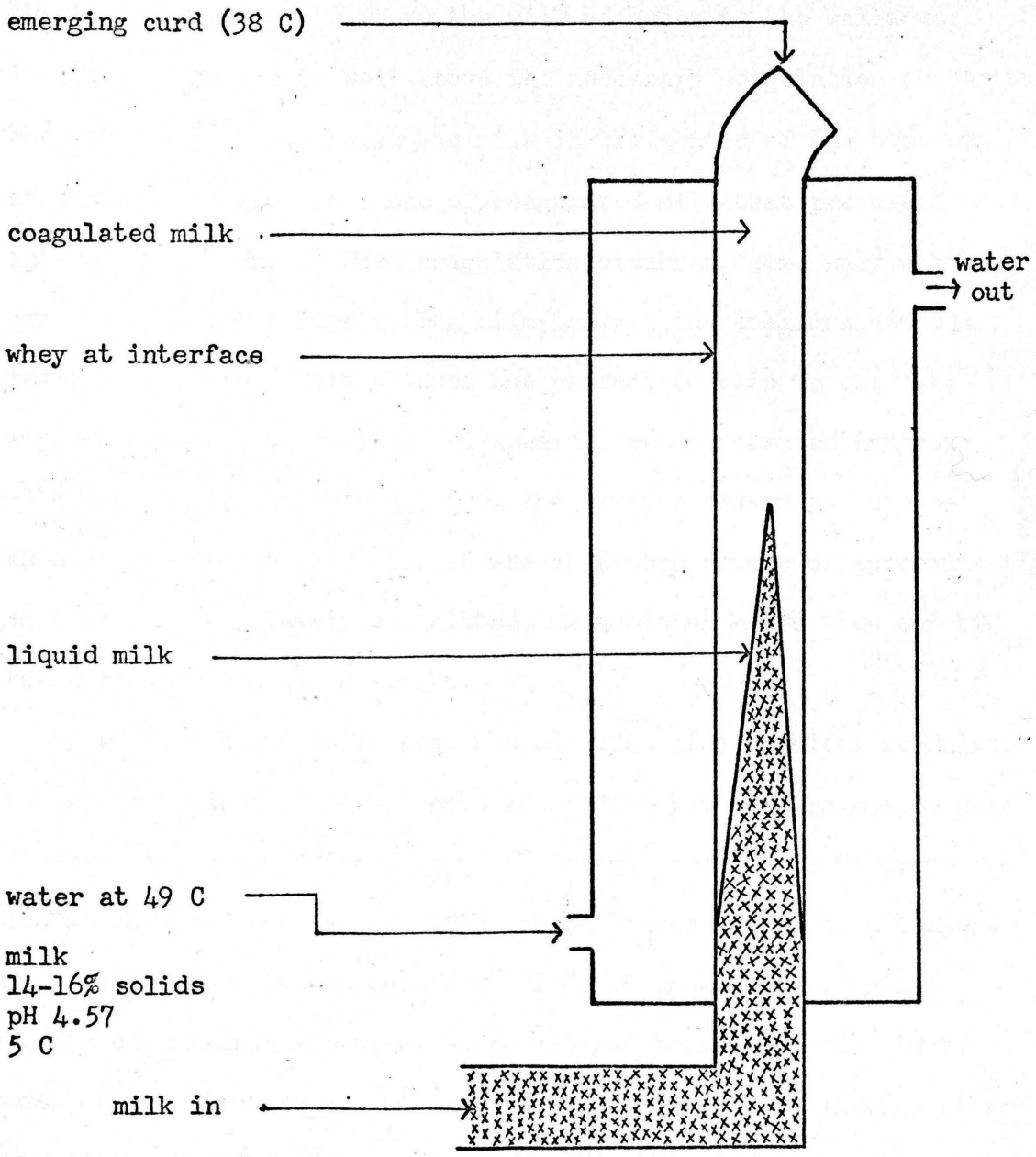

FIG. 1. Operational principles of the curd former.

an inside diameter of 3/8-inch was surrounded by a heating medium at approximately 49 C. The cold acidified high-solids milk entered the bottom of the tube where the milk adjacent to the walls was immediately heated to well above the necessary coagulation temperature. When this happened, the liquid milk in the center of the tube was effectively trapped in a sac of coagulated milk that prevented internal turbulence. When coagulation occurred, some whey was expelled to form a lubricating film between the coagulum and the wall of the tube. This allowed the product to slip up the tube without agitation. As it moved upward, heat penetrated into the milk causing it to coagulate from the outside toward the center. When it emerged from the top it was at a temperature of approximately 38 C and was completely coagulated. A rotating knife then cut off the curd at the desired length.

To evaluate the effect of the addition of a chemical acidulant to skimmilk, Ernstrom (10) found it necessary to determine the curd firmness and coagulation time of the coagulated caseinate system. It was observed that as acidified skimmilk was heated to a temperature where coagulation occurred (21 C) it took about 20 minutes to achieve maximum firmness. Curd tension tests indicated that most of the firmness was acquired during the first 10 minutes after the coagulation temperature was achieved, therefore all measurements of curd tension were made 10 minutes after the setting temperature was reached. Curd tension readings, recorded in grams, were taken at milk temperatures of 21 C and 25 C. Maximum firmness of the curd

was attained between pH 4.40 and pH 4.60 and the firmness of the curd at all pH values was higher at the higher setting temperature. In the continuous process, production of curd above pH 4.60 produced a product that tended to mat easily and a product that would not flow evenly through the curd former.

Advantages claimed by Ernstrom (11) for the continuous curd former are:

1. The total process time from cold acidified milk to the end of washings is 20-25 minutes.
2. The curd may contain 3-5 times as much lactose as that present in curd from conventional processes.
3. The extra lactose present means a greater recovery of milk solids which increases the yield.
4. A savings of 60-70% in labor costs should be realized.
5. The noncultured product would eliminate dead vats, slow vats, and lack of product uniformity due to variation in the behavior of cultures.

Ernstrom (10) pointed out that curd formed by chemical acidification does not now meet the Federal Standards of Identity for Cottage cheese. He felt that the product would probably be in the same category as sour cream made by acidification with citric or lactic acid. He did not believe direct acidification and continuous production would eliminate all Cottage cheese problems, but considered that the solution of a few of them would be worth while to the industry.

Effects of acidification on the growth of lactic culture bacteria. Smith (23) developed a procedure for determining the rate of acid production per cell of an actively growing lactic culture. A culture of Streptococcus lactis was incubated at 30 C in two liters of sterile, reconstituted nonfat milk for the length of the experiment. Fifty milliliter samples were titrated with 0.10 N sodium hydroxide to the phenolphthalein end point and the results were expressed as micro moles of acid per milliliter. The standard plate count was obtained by diluting 11 gram samples to yield plates containing 30 to 300 colonies. The number of bacteria present were expressed as cells per milliliter and were then plotted against the number of micro moles per milliliter to determine the micro moles of acid produced per cell.

Harvey (13) observed the damage to Streptococcus lactis resulting from growth at low pH values. He noted that growth of Streptococcus lactis at 30 C below pH 5.00 resulted in either direct inactivation of a number of enzymes, or in a decrease in the control of differential rates of synthesis of individual enzymes. When a pH shift was made from pH 6.30 to pH 4.50 the initial specific growth rate was higher than normal for pH 4.50. This transient growth rate was maintained for about 0.5 doubling of the bacterial cells, after which the normal growth rate for pH 4.50 was attained. When the transfer was from an initial pH of 4.20 to pH 5.20 or to pH 6.20, the specific growth rate immediately after the shift was less than normal for the final pH. This growth rate was maintained

for a short period, then gradually increased. The specific growth rate normal for the final pH was again attained after about 0.5 doubling of the cell mass. Changes in pH at a range of pH 5.00 to 6.30 were attributed only to effects of pH on the rate of the enzymatic reactions of the cell. Sudden changes within the limits of pH 5.00 to 6.00 resulted in immediate resumption of the growth rate specific for that pH level. Results indicated that the optimum pH for growth of Streptococcus lactis grown at 30 C was at pH 6.30. At pH 4.00 the specific growth rate approached zero. It was concluded that growth of Streptococcus lactis at pH values below 5.00 altered or damaged the cell in some manner which resulted in a decreased growth rate.

The physical character of the curd of milk is of primary importance with reference to the quality, texture, and body of many dairy products. The curd tension test aids in determining the curd making properties of the casein and appears to be valuable in predicting the quality of Cottage cheese. The curd character is one of the major factors in the production of quality cheese. The curd character may be affected by a number of factors, although time and temperature of heat treatment of skimmilk appear to be the dominating factors (24). Ernstrom (10) reported that the pH of the curd at cutting time was a factor in curd tension. The Department of Dairy and Food Industries at the University of Wisconsin advocated the use of a curd tension test for predicting the suitability of

nonfat dry milk solids for making Cottage cheese. These investigators found that Cottage cheese curd cut at pH 4.70 increased in quality as the curd tension of the coagulated caseinate system increased (5).

EXPERIMENTAL PROCEDURE

The effect of acidification of skimmilk on the acid producing ability of lactic starter bacteria and the quality of the resulting curd was studied. The method for determining the amount of acid produced per cell, expressed as micro moles of lactic acid, was derived from the work of Smith (23) and Harvey (13). Curd tension analysis was first proposed in Europe in 1916 (5) and was used for determining the elasticity of curd formed on coagulation. Originally this test involved human error since no mechanical means were used to drive the curd knife through the curd. Introduction of mechanically driven curd tension knives and direct reading of results have made this test a valuable tool in evaluating cultured dairy products.

Preparation of milk. The milk used throughout the experiment was reconstituted from Land O'Lakes pretested nonfat dry milk (NFDM) to 10% nonfat milk solids (116 grams NFDM to 1000 milliliters distilled water).

Preparation of cultures. Two cultures, E8 and H5, were used in this study. Culture H5 was a commercially lypholized mixed strain, multiple type culture obtained from Hansen's Laboratory, Milwaukee, Wisconsin. Culture E8 was a single strain Streptococcus cremoris culture originally isolated by Emmons (9) and described in his review of the manufacture of Cottage cheese. This culture was received

from the Food Science Department, Michigan State University, East Lansing, Michigan.

Reconstituted NFDM used for the transfer and storage of cultures was steamed for 45 minutes in a steam cabinet. To insure uniform activity, the experimental cultures were transferred daily for one week prior to the beginning of the experiment. During the experiment, stock cultures were transferred on a Monday, Wednesday and Friday schedule. The cultures were transferred using a 1% inoculation of the culture into steamed reconstituted NFDM tempered to 21 C. The inoculated skimmilk was incubated at 21 C for 14-18 hours. The cultures were removed from the incubator and placed in a refrigerator at 5 C for storage.

Preparation of samples. Duplicate 2 liter aliquots of reconstituted NFDM, cooled to 5 C, were placed in stainless steel vats 6 inches wide x 6.5 inches long and 6.5 inches deep. Hydrochloric acid (2.0 N), at 5 C was added to bring one of the milk samples to the desired pH. The quantity of acid required was determined from the titration curve presented in Figure 2. The acid and milk were thoroughly mixed before the culture was added. A 5% inoculation was used in the experimental vat and the milk was allowed to reach the incubation temperature of 32 C in a quiescent state. The second vat was maintained as a control and also received a 5% inoculation of the desired culture and an amount of water equal in volume to the acid added to the experimental vat. The addition of water to the

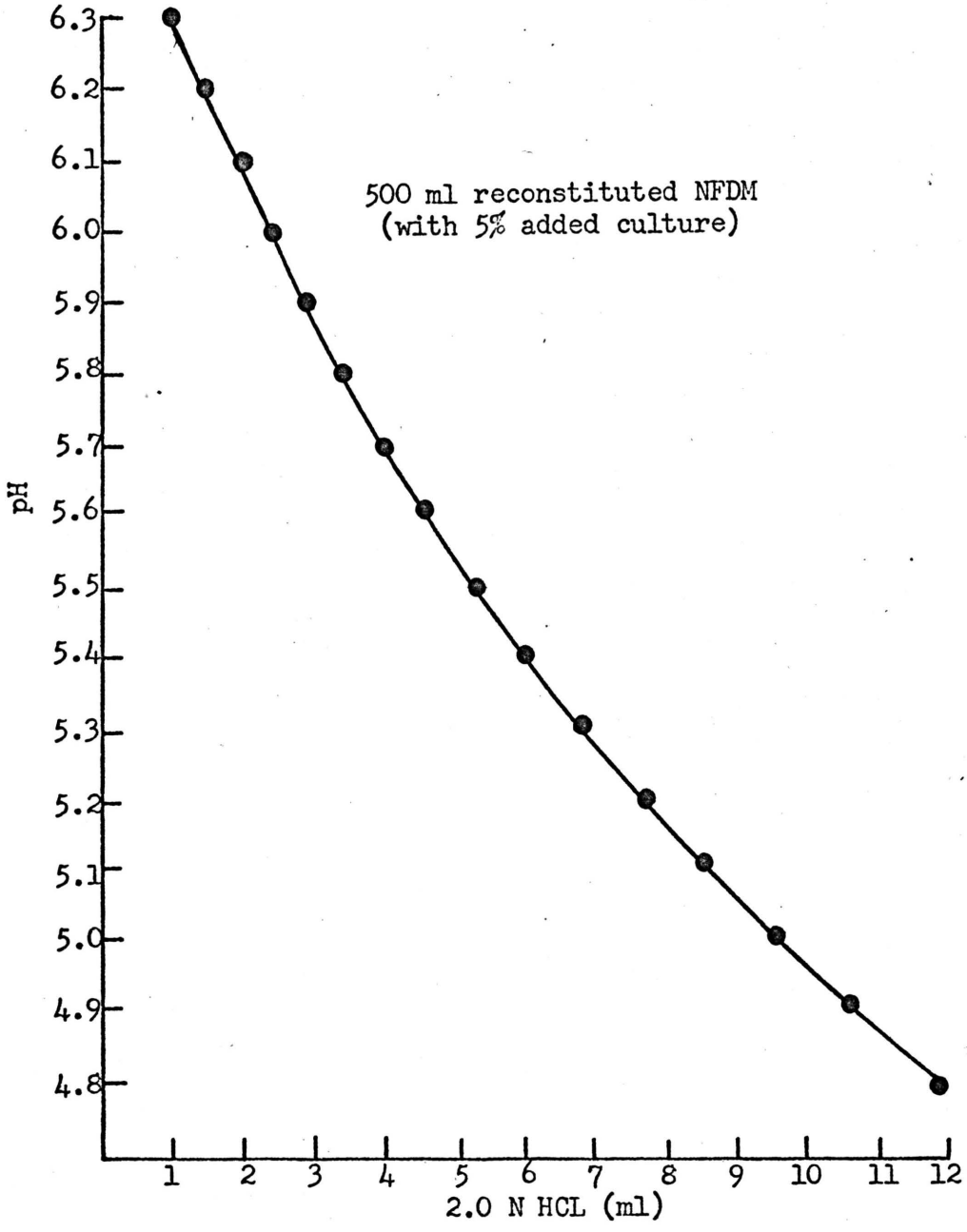

FIG. 2. Titration curve for addition of 2.0 N HCL.

control vat eliminated the effect of any dilution of the caseinate system which might occur in the experimental vat due to the addition of the hydrochloric acid.

Samples for the total plate count and titratable acidity test were taken at the beginning of the experiment and at hourly intervals throughout the remainder of the experiment. All samples to be tested were removed from the vat with a 17.6 milliliter Babcock milk pipette to minimize agitation and dispersion of the curd. Collection of the samples for the total plate count and titratable acidity test were terminated when the respective vats reached the value of pH 4.70, which is the isoelectric point of casein and is the pH used to derive the cutting time in a normal Cottage cheese operation. The total time in minutes required for the preacidified samples to reach pH 4.70 was recorded. This value was compared to the time recorded for the control vat to determine if a decrease or increase in coagulation time occurred.

Bacteriological analysis. The standard plate count procedure for milk (25) was used to determine the bacterial population. The total count was obtained at zero time (time of inoculation) and at hourly intervals thereafter until the samples reached a final pH of 4.70. Since the presence of a 5% starter culture implicates a large number of bacteria, dilutions of 1×10^{-4}, 1×10^{-5} and 1×10^{-6} were used for hours 0 through 3, dilutions 1×10^{-6}, 1×10^{-7} and 1×10^{-8} were used at 4 hours and after. Duplicate plates were poured using dilutions occurring in the middle of each range. Standard Methods

agar (Difco 0479-01) (8) was used and the plates were incubated at 32 C for 48 hours. A dark-field Quebec colony counter was used for counting the colonies of bacteria. The count per milliliter in each case was determined by multiplying the number of colonies counted by the dilution of the individual plate.

Chemical analysis. The titratable acidity test for cultured dairy products (25) was performed simultaneously with the standard plate count to determine the total amount of acid, calculated as lactic acid, produced by the bacterial cells at hourly intervals. A 17.6 milliliter Babcock milk pipette was used to obtain the sample and the sample was then diluted with twice its volume with carbon dioxide-free water. The sample was titrated with 0.1 N sodium hydroxide to the phenolphthalein end-point of pH 8.30 (8). A Corning Model 7 pH meter equipped with a Sargent Model S-30072-15 combination glass electrode was used to verify the end point.

Physical analysis. Curd tension readings, measured in grams, were taken at pH 4.70 of samples preacidified from pH 6.20 through pH 4.80. The samples of reconstituted low-heat NFDM were prepared in 500 milliliter aliquots and placed in a 600 milliliter beaker and cooled to 5 C. All acidified samples and a control sample received a 5% inoculation of the desired culture and the desired pH was reached by adding 2.0 N hydrocholoric acid according to the titration curve presented in Figure 2. The samples were placed in a 32 C water bath and incubated until a pH of 4.70 was reached. Curd

tension was measured with the apparatus exhibited in Figure 3. The original piece of equipment was obtained from the Submarine Signal Company, Boston, Massachusetts and was designed to determine the curd tension of processed Cottage cheese curd.

The original apparatus was modified to increase the precision of the results obtained from freshly coagulated curd. An arm extending from the motor to the scale was designed to place constant, evenly distributed pressure on the scale pan. The scale used for this experiment had twice the sensitivity of the measuring device originally installed on the machine. To insure adequate reading from the experimental samples it became necessary to design a special curd tension knife having an increased surface area. The knife was constructed of stainless steel and is shown in Figure 4. The base of the knife was 2 inches square with 9 evenly spaced blades to make up the cutting area. The shaft of the knife was parallel to the outer edge of the knife base. This feature prevented the addition of pressure by the shaft upon the surface of the curd as the knife was driven down into the curd.

The sample to be tested was placed on the scale pan. The curd knife was driven at a constant, controlled rate of 1 inch per 7 seconds by a precision built synchronous Submarine Signal motor. The readings were taken directly from the scale and were observed when the resistance offered by the curd was constant. The scale had a capacity of 500 grams and was graduated in 1-gram divisions so measurements could be interpreted to the nearest 1/2-gram.

FIG. 3. Apparatus for determining curd tension.

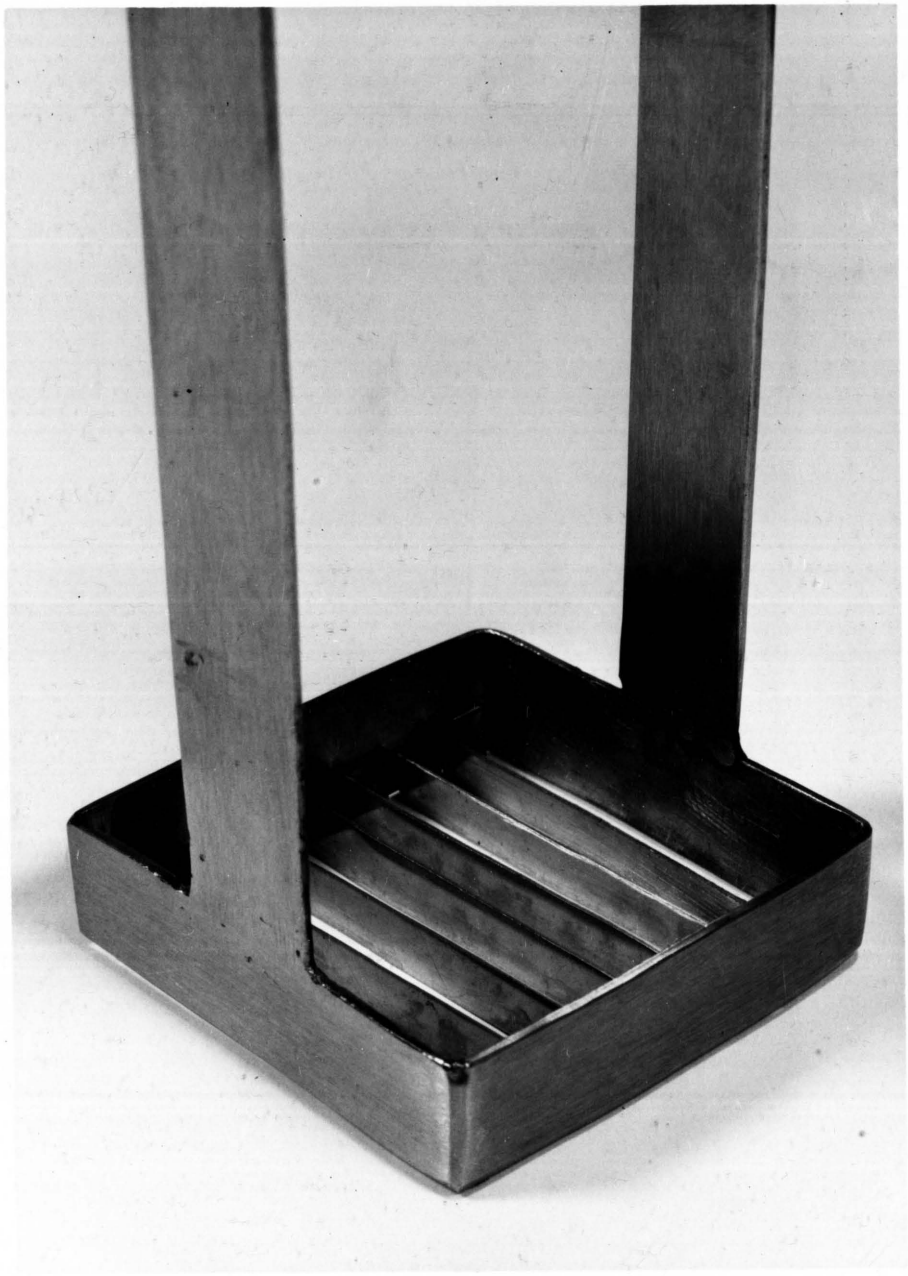

FIG. 4. Specially designed curd tension knife.

RESULTS AND DISCUSSION

The primary attempts to reduce the coagulation time of the caseinate system of skimmilk for Cottage cheese manufacture have dealt with either an increase in starter inoculation or with a continuous direct acidification method. These procedures have resulted in a decrease in manufacturing time for Cottage cheese. The use of increased inoculation adversely affected the body and texture of the finished product (17). The use of chemical acidulants involved the use of continuous curd formers and would not make use of equipment now available in the cheese plant (11,14). The combined use of a chemical acidulant and starter bacteria could therefore possibly result in a process that would:

1. Decrease the coagulation time.
2. Make use of conventional equipment.
3. Produce a product equal in quality to that made by conventional methods.

Because of Federal Standards of Identity for Cottage cheese, this product cannot now be legally produced by an acidification method. However, advocates of the direct acidification method can receive temporary permits to manufacture Cottage cheese by this method to study the merits of the process.

The major deterrent to increased production in a normal cheese producing operation has been that each cheese vat was used for 8 or more hours a day for each lot of cheese produced. A period of 4 to

5 hours is usually required for the setting process. Also, many of the defects which frequently occur in the final product develop during the setting of the curd and can often be attributed to the starter bacteria. Research workers desiring to decrease contamination and increase production by greater utilization of existing plant equipment have tried to decrease the setting time of Cottage cheese curd.

Decrease in coagulation time due to acidification. Duplicate 2 liter aliquots of reconstituted NFDM cooled to 5 C were placed in a small Cottage cheese vat. The cultures studied were E8, a single strain Streptococcus cremoris, and H5 a mixed strain multiple type culture. One experimental vat was used as a control vat and was given a 5% inoculation of the desired culture. The second experimental vat was inoculated with 5% of the desired culture before the sample was acidified to the desired pH with 2.0 N hydrochloric acid. The pH levels used were pH 6.25, 6.00, 5.75, 5.50, 5.25 and 5.00. The vats containing the inoculated samples were suspended in a water bath and were allowed to reach the incubation temperature of 32 C. The optimum pH for cutting Cottage cheese curd is approximately pH 4.70 (12). Therefore pH 4.70 was selected as the end point of incubation throughout the experiment. The time required for the experimental culture to change the milk from the pH at inoculation to pH 4.70 was determined. When compared to the control sample the time recorded for the acidified sample indicated the decrease in time in coagulation attributed to acidification.

TABLE 1

Influence of acidifying milk to different pH values on setting time for Cottage cheese curd.

Acidified sample pH	E8 Culture			H5 Culture		
	Coagulation time (Minutes)		% Decrease*	Coagulation time (Minutes)		% Decrease*
	Control	Acidified		Control	Acidified	
6.25	290	270	6	300	285	5
6.00	330	290	12	300	275	10
5.75	315	270	11	310	280	10
5.50	315	300	5	300	285	5
5.25	315	285	10	310	270	12
5.00	300	180	40	300	150	50

*Decrease in coagulation time resulting from acidification.

From the results in Table 1, very little variation in coagulation time seems to exist between cultures E8 and H5. Acidification of the milk to pH 6.25 and pH 5.50 caused the least decrease in coagulation time. Acidification to a pH of 6.25 would not be expected to decrease the coagulation time to any great extent since this value is only 0.20-0.30 pH units below that of the normal pH of milk. Acidification to pH 5.50 did not show as great a decrease as did acidification to the other pH values. This could be attributed to inactivation of certain enzymes functioning at this pH. No appreciable difference in coagulation time occurred between milk acidified to pH 6.00, 5.75 and 5.25. The greatest decrease in time (40-50%) was observed from acidifying the milk to pH 5.00 prior to adding the culture.

The results for coagulation time of the control samples for E8 and H5 show that culture H5 did not vary in the setting time to as large a degree as did culture E8. This would indicate that the mixed strain multiple type culture was more stable and produced more consistent results when compared to the single strain E8 culture.

Attempts to decrease setting time for Cottage cheese manufacture have been limited to increasing the rate of inoculation to various levels from 5% to 20%. Reports of increased inoculation (above 5%) showed that a decrease in the quality of the body and texture often occurs. Excessive amounts of starter culture add to the quantity of denatured protein present in the skimmilk and tend to interfere with the "good knitting" of Cottage cheese curd. Hales (12) observed that over the common range of 5% to 8% starter used in the short time method, the reduction in time required to reach pH 4.70 amounted to only 10 to 15 minutes, for every 1% increase of starter. He found that the overall difference in time between 5% and 10% added starter was less than 1 hour. The increased inoculum added a considerable cost factor and put an increased emphasis on starter bacteria for acid production.

The results in Table 1 were obtained without an increase in percent starter culture. This procedure would also decrease the emphasis placed on starter bacteria for acid production. Hales (12) observed that a decrease of 1 hour would not be an economic savings when an increase of 5% to 10% added starter was used. Acidification to pH 5.00 decreased the time of coagulation for both E8 and H5 culture by at least 2 hours. Without the additional cost

of an increased starter culture acidification to this pH should decrease coagulation time sufficiently to make it applicable to Cottage cheese manufacture.

The effect of acidification on acid production of starter bacteria. Smith (23) studied growth and acid production of lactic cultures. He believed that when studying acid production in lactic cultures, it would be helpful to be able to determine if the rate of acid production per cell was changed by the presence of acidulants, if the growth rate was affected, or if both were altered.

The data for this experiment were collected at hourly intervals from acidified milk samples with 5% added culture (Table 1). The bacteriological analysis was performed by weighing 11 grams of sample into a sterile 99 milliliter dilution blank. The appropriate dilutions were prepared to obtain counts between 30 and 300 colonies per plate. The plates were incubated at 32 C for 48 hours. The titratable acidity test was performed to determine the total amount of acid, calculated as lactic acid, produced by the bacterial cells at hourly intervals. A paired Student's t test (26) was performed to compare the control and sample means within each culture and pH level. The hypothesis that the mean of the differences ($\bar{d}$) of the paired variates was zero was tested. The 0.01 level of probability was selected to indicate a real difference.

Table 2 indicates the t comparisons for acidified and control samples ranging from pH 6.25 to pH 5.00. At pH 6.25 culture E8 showed a significant difference between control and sample means.

TABLE 2

Student's t analysis of results of acidification of skimmilk on acid production by cultures E8 and H5

Acidified Sample pH	df[a]	t[b]	E8 Culture			H5 Culture			
			Control $\bar{x}$	Sample $\bar{x}$	$\bar{d}$[c]	t	Control $\bar{x}$	Sample $\bar{x}$	$\bar{d}$
			1×10^{-6}	uM/cell/hr			1×10^{-7}	uM/cell/hr	
6.25	15	3.341**[d]	1.5710	0.3540	1.217	4.767**	0.4012	1.3557	0.956
6.00	13	0.874NS[e]	0.3601	0.3205	0.039	6.169**	1.9878	1.0685	0.919
5.75	10	0.760NS	0.2650	0.2506	0.014	2.307*	1.3560	0.8412	0.515
5.50	8	2.795*[f]	0.4783	0.3656	0.112	2.036NS	0.1814	0.1258	0.055
5.25	5	1.168NS	0.1363	0.1712	0.035	1.103NS	1.9500	1.8540	0.016
5.00	3	4.303*	0.4675	0.1770	0.291	1.848NS	0.0825	0.1930	0.110

[a]Degrees of freedom.
[b]Student's t test.
[c]Mean of the differences.
[d]Highly significant P< 0.01.
[e]Not significant.
[f]Significant P< 0.05.

At pH 6.00, 5.75, 5.50, 5.25 and 5.00 the differences were not significant. The control and sample means were quite similar and the $\bar{d}$ values were well below those of the other pH levels tested.

Culture H5 showed varying results from the E8 culture. At pH 6.25 and pH 6.00 the control and sample means for culture H5 were significantly different. It should be noted that although the control and sample means varied among themselves at these pH levels the $\bar{d}$ value for the two pH levels was very close. Acidification to a pH of 5.75 and below indicated no significant differences at the 0.01 level. This would indicate pH did not affect acid production at these pH levels.

Harvey (13) studied the effect of pH on the growth of Streptococcus lactis cells. He observed that growth at pH 5.00 or below altered or damaged the cell in some manner and decreased the growth rate. When shifts from high to low pH occurred in the range of pH 6.00 to pH 5.00, resumption of the growth rate specific for that range was obtained immediately. When a shift up in pH occurred at pH 5.00, and below, the specific growth rate normal for the final pH was not obtained until about 0.5 doubling of the cell mass. The optimum pH for growth of Streptococcus lactis grown at 30 C was at pH 6.30. At pH 4.00 the specific growth rate for Streptococcus lactis approached zero. When comparing the effect of pH on Streptococcus lactis, to the data in Table 2, we find that with cultures H5 and E8 acid production did not decrease until after pH 5.25 was

reached. Harvey (13) indicated this decrease could be a result of damage to, or complete inactivation of, enzymes active at this pH.

Smith (23) studied the acid producing ability of Streptococcus lactis grown at 30 C in reconstituted NFDM. Inspection of a semilogarithmic plot of cells versus sampling time indicated that the culture exhibited logarithmic growth from the second through the sixth hour. A regression analysis line indicated that the relationship between cell numbers and acid production was linear. The rate of acid production per cell was calculated to be 7.46×10^{-9} micro moles of lactic acid per cell per hour for those cells exhibiting logarithmic growth. The value for acid production per cell observed by Smith (23) agrees closely with the range of 0.1258×10^{-7}-1.5710×10^{-6} micro moles of lactic acid obtained when cultures E8 and H5 were studied.

The effect of acidification on curd tension. The physical character of the curd of milk is of primary importance with reference to the quality, texture, and body of many dairy products. The curd tension test determines the curd making properties of the casein. This test is valuable in predicting the quality of finished Cottage cheese. Samples of reconstituted low-heat NFDM were prepared in 500 milliliter aliquots, placed in a 600 milliliter beaker, and cooled to 5 C. Curd tension readings were taken of samples acidified from pH 6.20 through pH 4.80 at 0.1 pH intervals. The desired pH of the acidified sample was obtained by the addition of 2.0 N hydrochloric

acid. All acidified samples and a control sample received a 5% inoculation of the experimental culture. The data in Table 3 indicate the results obtained from the Student's t analysis of data for cultures E8 and H5.

When examining the significance occurring from samples acidified to pH values below pH 6.00, a definite difference between control and sample means seems to exist when culture E8 was used. There was no significant difference between control and sample observations in samples acidified to pH 6.20, 6.10 and 6.00. The $\bar{d}$ for these 3 values was considerably lower than the $\bar{d}$ of any of the other samples tested. This would indicate the difference between control and sample means due to effect of acidification was lowest with milk acidified to pH 6.20, 6.10 and 6.00. At milk acidified to pH 6.10 the control mean and sample mean values were identical. The curd tension from milk acidified to pH 6.00 was the highest (29.33 grams) sample mean of all the samples which showed no significant difference from the control. The curd tensions of control versus sample means for all milk acidified below pH 6.00 were significantly different at the 0.01 levels.

The lowest sample mean of 17.91 grams of curd tension was recorded with milk acidified to pH 5.50. The low sample mean indicates acidification of milk to pH 5.50 adversely affects curd tension. The abnormally high $\bar{d}$ value (16.33 grams) recorded for curd from milk acidified to pH 5.70 did not seem to fit in with the

TABLE 3

Student's t analysis of acidification of skimmilk on curd tension produced by E8 and H5

Acidified Sample pH	E8 Culture				H5 Culture			
	Curd tension				Curd tension			
	t[a]	Control $\bar{x}$[b]	Sample $\bar{x}$	$\bar{d}$[c]	t	Control $\bar{x}$	Sample $\bar{x}$	$\bar{d}$
	Grams				Grams			
6.20	0.338NS[d]	25.92*	25.67	0.25	2.153NS	17.42	20.00	-2.58
6.10	0.000NS	24.92	24.92	0.00	1.469NS	23.92	20.83	3.08
6.00	0.506NS	28.42	29.33	0.92	2.904*[f]	20.17	15.83	4.33
5.90	8.468**[e]	36.25	29.25	7.00	10.403**	22.42	18.00	4.42
5.80	21.152**	30.17	23.25	6.92	4.227**	17.17	15.59	1.58
5.70	11.700**	38.17	21.92	16.33	2.764*	21.83	17.92	3.92
5.60	15.108**	27.10	20.50	6.66	3.514*	19.67	16.50	3.16
5.50	14.587**	26.00	17.91	8.08	0.000NS	16.75	16.75	0.00
5.40	8.396**	36.00	25.16	10.92	5.726**	20.58	16.00	4.58
5.30	12.177**	31.33	18.58	12.75	6.198**	14.00	11.42	2.58
5.20	9.69**	33.08	20.92	12.17	2.379NS	17.33	14.17	3.17
5.10	26.62**	33.92	20.75	13.17	5.644**	25.66	19.00	6.67
5.00	7.78**	29.67	19.67	10.00	12.969**	17.92	13.75	4.33
4.90	14.43**	28.58	18.17	7.08	10.202**	25.08	21.50	3.58
4.80	6.937**	33.75	20.50	13.41	4.533**	17.42	13.25	4.17

[a]Student's t test.
[b]Mean of 6 observations.
[c]Mean of the differences.
[d]Not significant.
[e]Highly significant P< 0.01.
[f]Significant P< 0.05.

other observed values. When reviewing the data it was observed that the control mean for curd tension of milk acidified to pH 5.70 was an unusually large value of 38.17 grams compared to 21.92 grams for the sample mean at this pH. This abnormally high control mean value tended to increase the $\bar{d}$ above that of milk acidified in surrounding pH levels.

The curd tension analysis for culture H5 revealed differing levels of significance when compared to the corresponding levels of significance of culture E8. Milk acidified to pH 6.20, 6.10 and 6.00 resulted in no significant differences when both E8 and H5 were tested. Milk acidified to pH 5.90 and pH 5.80 resulted in $\bar{d}$ values having significance at the 0.01 level, while milk acidified to pH 5.70 and pH 5.60 were significant only at the 0.05 level. Generally milk acidified to pH 5.40-4.80 showed a significant difference in the curd tension of the control and sample means. However milk acidified to pH 5.20 resulted in control and sample means showing no significant difference level. The observation of particular interest in the analysis of curd tension produced by culture H5 was that the control and sample mean values were consistently lower than the values obtained from culture E8. Also the $\bar{d}$ values for culture H5 were consistently lower when milk was acidified below pH 6.00. This would indicate that the curd tension producing properties of this culture were not as subject to the adverse affects created by acidification.

The data presented in Table 3 for the control and sample means for both E8 and H5 cultures is an average of 6 individual observations recorded at the various pH levels. A regression analysis (26) of curd tension on pH was performed on the observations to determine whether meaningful regression equations for curd tension, with variation in pH, were possible. The $\underline{Y}$ variable is termed the dependent variable and the $\underline{X}$ variables are the independent variables. The equations for the regression lines presented in Figure 5 are as follows:

Culture E8

$$\text{Control } \underline{Y} = 313.4 + (-5.5094)\ (\underline{X}-8) + .3536(\underline{X}^2 - 82.666)$$

$$\text{Sample } \underline{Y} = 231.77 + (-6.5972)\ (\underline{X}-8) + .5607(\underline{X}^2 - 82.666)$$

The equations for the regression lines presented in Figure 6 are as follows:

Culture H5

$$\text{Sample } \underline{Y} = 166.77 + (-5.1366)\ (\underline{X}-8) + .4274(\underline{X}^2 - 82.666)$$

$$\text{Control } \underline{Y} = 197.77 + (+7.5625)\ (\underline{X}-8) + -.8702\ (\underline{X}^2 - 82.666)$$

The $\underline{X}$ values substituted into the equations were the various pH values tested. These values were coded by subtracting from each pH value (pH 6.20-4.80) the quantity of 4.70. This value was multiplied by a factor of 10 to give the coded $\underline{X}$. This gave pH 6.20 an $\underline{X}$ value of 15 with all subsequent pH units having a correspondingly decreased $\underline{X}$ value. The values obtained from the regression equations had to be decoded by decreasing their value by a factor of 10 units.

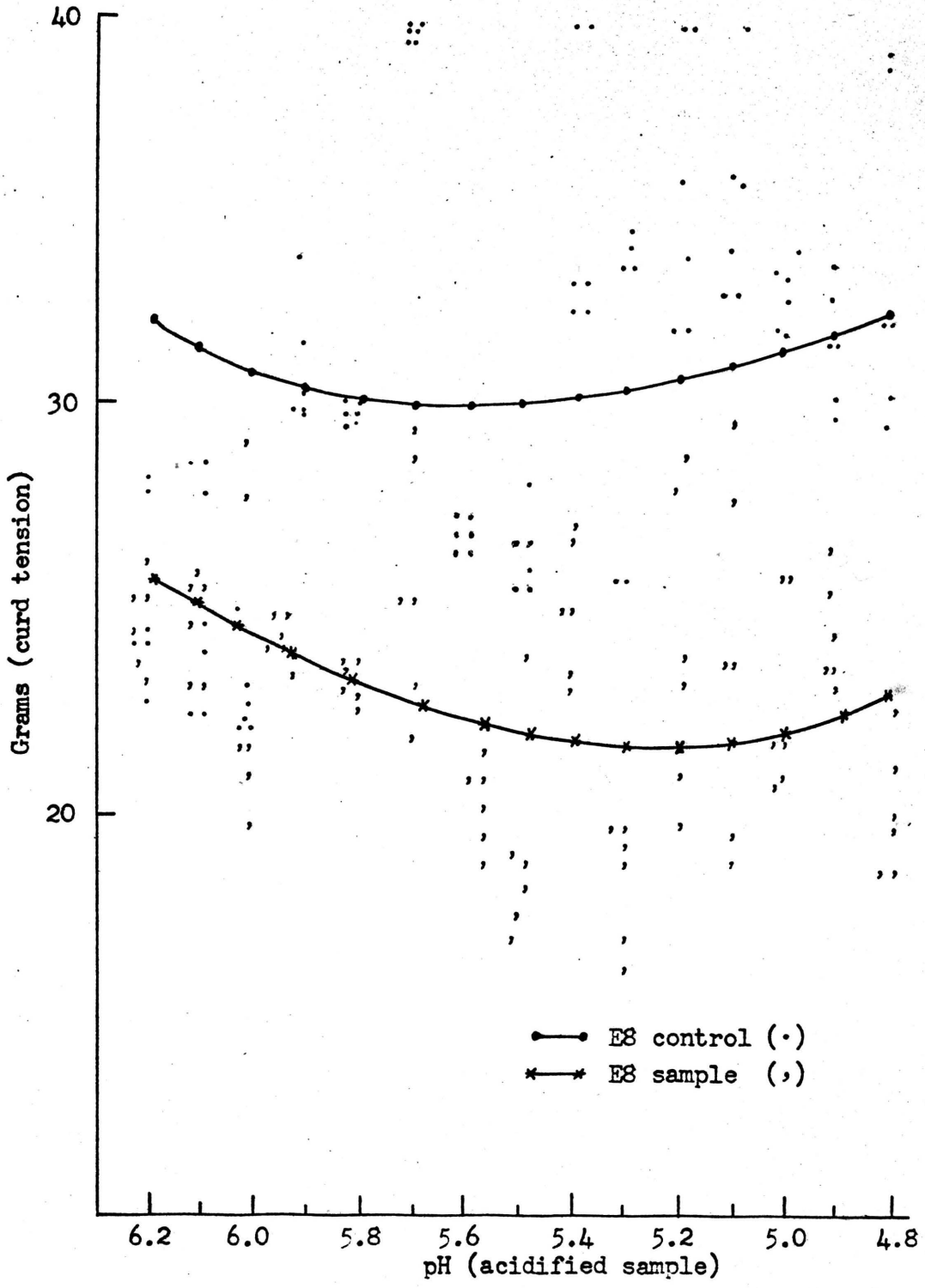

Fig. 5. Regression of curd tension produced on pH for E8 culture.

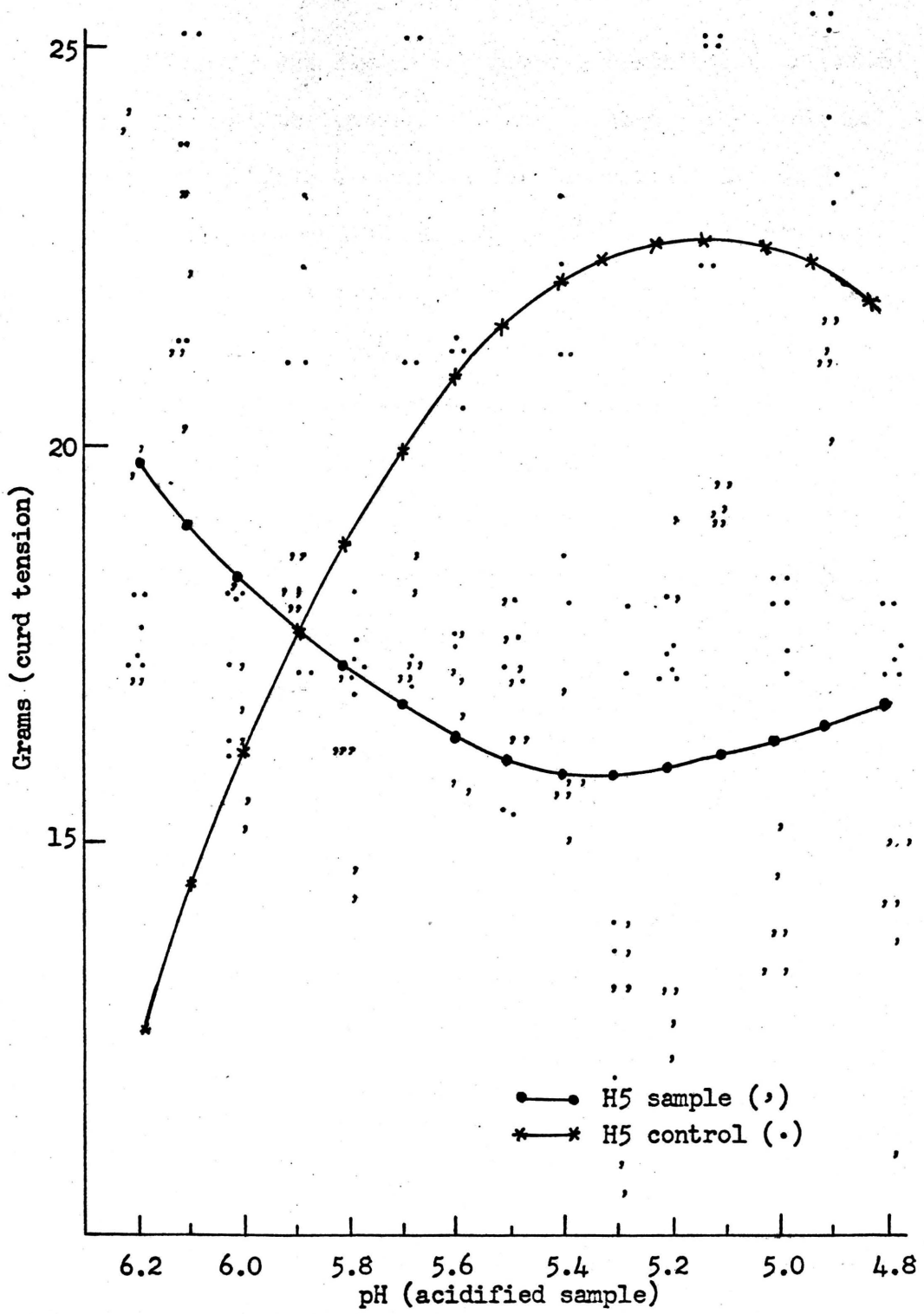

Fig. 6. Regression of curd tension produced on pH for H5 culture.

Included in Figure 5 and Figure 6 are the 6 observations recorded at each pH level for both the control and sample used. The analysis of variance recorded in Table 4 accounts for the variability of Y. The regression line for E8 control was not significant, therefore we could not account for a significant portion of the variability in Y. The regression lines for E8 sample, H5 control and H5 sample were significant and the equations presented indicated that varation in X accounted for a significant amount of the variability of Y. In all cases extending the equation to a cubic did not account for sufficient variability, therefore the quadratic equation was used. The variability of the observations exists from acidified pH to acidified pH rather than within pH as might be expected. This variability would limit the usefulness of the curves in their ability to predict future results. No attempt was made to compare the control and sample lines for either E8 or H5 culture.

It has long been established that curd tension is a physical characteristic of primary importance in cultured dairy products. Workers at the Department of Dairy and Food Industries at the University of Wisconsin found that when Cottage cheese curd was cut at pH 4.70 the quality of the finished product increased with the tension of the curd (5). On the basis of this information a comparison of the t values with the sample means indicate that those t values which were not significant also were the values with the largest sample mean values. This would mean accidification of skimmilk to these pH values would produce a curd with a small degree of variance and a curd with sufficient strength to produce a suitable finished Cottage cheese.

TABLE 4

Analysis of variance for the regression of curd tension on pH

Source	df	E8 control SS	E8 control MS	E8 sample SS	E8 sample MS
Due to regression	2	3,132.7	1,566.4	17,329.1	8,664.6
Linear	1	37.2	37.2 NS	9,547.6	9,547.6**
Quadratic	1	3,095.5	3,095.5 NS	7,781.5	7,781.5**
Error	87	122,672.8	1,410.0	73,786.4	848.1

Source	df	H5 control SS	H5 control MS	H5 sample SS	H5 sample MS
Due to regression	2	86,699.7	43,349.8	9,391.1	4,695.6
Linear	1	67,957.9	67,957.9**	4,868.8	4,868.8*
Quadratic	1	18,741.8	18,741.8**	4,542.3	4,542.3*
Error	87	211,391.7	2,429.8	84,618.9	972.6

SUMMARY AND CONCLUSIONS

Direct acidification of dairy products was started on a commercial scale in 1962. Sour cream was the first product produced by this method. During the period following 1962 the direct-acid process for sour cream has been used extensively. The direct-acid process has established itself as superior to culturing in many respects, and is now being used in most states where it is approved.

Labeling of acidified products has been one of the largest obstacles to overcome. One of the reasons for this is that the process is new and a radical departure from conventional techniques. The Federal Food and Drug Administration has no standard of identity for sour cream. The United States Public Health Service defines sour cream in such a way that it can be produced either by the culturing process or by direct acidification. Because of federal standards for Cottage cheese, this product is in a different category than sour cream. In order to study the merits of direct acidification, the Food and Drug Administration announced they would be receptive to application for temporary permits to manufacture Cottage cheese by direct acidification. The Creamery Package Manufacturing Company has secured a permit for manufacture of Cottage cheese by the Ernstrom (11) process. They have also obtained a permit for sale of the product in Wisconsin and Illinois. It is logical to presume that if and when these processes prove their merit in commercial application, the Food and Drug Administration will be

receptive to amending the standards to permit their use in Cottage cheese manufacture.

The continuous processes of Ernstrom's (11) and Lankford's (14) were modified to be used in a vat operation. The procedure was to use a culture bacteria in combination with a chemical acidulant to decrease the time required for formation of the curd. A single strain E8 culture and a mixed strain multiple type H5 culture were investigated to determine their application to an acidified process. The combination of acidulant and starter bacteria was investigated to determine:

1. If the coagulation time of the caseinate system could be reduced.
2. The effect of acidulants on acid producing ability of the bacterial cell.
3. The effect of acidulants on the curd tension of the coagulated caseinate system.

A decrease in coagulation time could be accomplished by acidification of milk prior to adding culture. Most pH levels tested showed a decrease in time ranging from 5 to 12% while acidification to pH 5.00 decreased coagulation time by as much as 40 to 50%. The type of culture used, single strain or mixed strain multiple type, did not appear to vary the results obtained.

The effect of acidification on the acid producing ability of E8 and H5 cultures was studied. A student's t analysis indicated that a highly significant difference between control and sample means was

only observed when skimmilk was acidified to pH 6.25 and pH 6.00 for culture H5. Culture E8 also showed a highly significant difference between means at pH 6.25. Results indicated that acidification to levels below pH 6.00 did not adversely affect the acid production of the bacterial cells present at the 0.01 level of testing. Culture E8 consistently produced a greater quantity of acid per cell when compared to the acid production of culture H5.

The effect of acidification on the curd tension of the caseinate system was studied. A student's t analysis suggested definite differences between control and sample observations for curd tension produced by E8 and H5 cultures at the various pH levels. Acidification below pH 6.00 adversely affected curd tension when culture E8 was used, however H5 showed no adverse effects from acidification until pH 5.40 and below. Culture E8 consistently exhibited curd tension readings above those produced with H5 culture.

A regression analysis of curd tension on pH was performed on the observations to determine whether meaningful regression equations for curd tension, with variation in pH, were obtainable. An analysis of variance indicated that variation in X accounted for a significant portion of the variability in Y when H5 control, H5 sample and E8 sample were tested. The regression line for E8 control was not significant.

To further understand the complete acidulant process an experimental vat of Cottage cheese was made using the process

fresh skimmilk at 5 C was fortified with NFDM, and acidified to pH 4.70 with 2.0 N hydrochloric acid. The acidified milk was heated to the incubation temperature of 32 C and allowed to coagulate in a quiescent state. No difficulties were encountered in the formation of the curd from the acidified skimmilk. The final product possessed a bland, clean acid flavor, and some shattered curd was observed. Observations of the finished Cottage cheese indicated a satisfactory product could be produced using the acidifying process.

From the results obtained in this experiment it appears acidification of skimmilk prior to the addition of culture can produce satisfactory Cottage cheese. To better utilize plant space and equipment, Cottage cheese processors may find this procedure helpful in producing a quality product.

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